Influence of glutathione-S-transferase (GSTM1, GSTP1, GSTT1) and cytochrome p450 (CYP1A1, CYP2D6) polymorphisms on numbers of basal cell carcinomas (BCCs) in families with the naevoid basal cell carcinoma syndrome

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ELECTRONIC LETTER

Influence of glutathione-S-transferase (GSTM1, GSTP1, GSTT1) and cytochrome p450 (CYP1A1, CYP2D6) polymorphisms on numbers of basal cell carcinomas (BCCs) in families with the naevoid basal cell carcinoma syndrome

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Background: The naevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant multisystem disorder with variable expression. NBCCS patients have variable susceptibility to development of basal cell carcinoma (BCC). Previous studies have shown that polymorphisms of some metabolic genes encoding the cytochrome p450 (CYP) and glutathione-S-transferase (GST) enzymes influenced the numbers of BCCs in sporadic BCC cases.

Objective: To determine whether allelic variants of these genes contribute to the variation in numbers of BCCs observed in NBCCS families.

Methods: Genotyping and analysis was carried out in 152 members (69 affected and 83 unaffected) of 13 families with NBCCS for seven polymorphisms in five metabolic genes including CYP1A1, CYP2D6, GSTM1, GSTP1, and GSTT1.

Results: GSTP1 Val^{105} and GSTP1 Val^{114} alleles were significantly associated with fewer BCC numbers (odds ratio $(OR)_{105} = 0.55$ (95% confidence interval, 0.35 to 0.88); $OR_{114} = 0.20$ (0.05 to 0.88)). The Val^{105} allele showed a dose dependent effect ($OR_{Ile/Val} = 0.58$ (0.34 to 0.88); $OR_{Val/Val} = 0.34$ (0.14 to 0.78)). In addition, fewer jaw cysts were observed in carriers of the three p450 polymorphisms (CYP1A1m1, CYP1A1m2, and CYP2D6*4) ($OR_{CYP1A1m1} = 0.27$ (0.12 to 0.58); $OR_{CYP1A1m2} = 0.25$ (0.08 to 0.78); $OR_{CYP2D6*4} = 0.33$ (0.18 to 0.60)).

Conclusions: Genetic variants might contribute to the variation in numbers of BCCs and jaw cysts observed in NBCCS families.

The naevoid basal cell carcinoma syndrome (NBCCS), also know as Gorlin syndrome, is a rare autosomal dominant disorder characterised by basal cell carcinomas (BCCs), odontogenic keratocysts of the jaws, pits of the palms and soles, ectopic calcification particularly of the falx cerebri, and skeletal anomalies. Other features, including ovarian fibromas, medulloblastoma, ocular anomalies, and neurological defects, are also associated with this syndrome.[1 2] The prevalence of NBCCS is about 1 per 60 000.[3] It arises in all races and has similar occurrence in males and females.[4] A majority of families possesses detectable mutations in the human homologue of the Drosophila patched gene, PTCH1, which is located on chromosome 9q22.3.[5 6] PTCH1 is a tumour suppressor gene, encoding a transmembrane glycoprotein that acts as an antagonist in the Hedgehog signalling pathway.[7] Mutation of PTCH1 also occurs in relevant sporadic tumours such as BCC, medulloblastoma, and trichoepithelioma.[2 8] More than 60 PTCH1 mutations have been reported in association with NBCCS, with the majority leading to premature truncation of the PTCH1 protein.[9] So far, no link has been found between different PTCH1 mutations and the phenotypic manifestations of NBCCS. Unrelated NBCCS patients carrying the same mutation have been found to present varying phenotypes.[10] Similarly, NBCCS kindreds with similar or identical mutations differ dramatically in the expression of clinical features.[8 10 11] These findings suggest that genetic background or environmental exposures, or both, may play important roles in modifying the phenotypic expression of NBCCS.

NBCCS patients have varied susceptibility to BCC development, the number of lifetime BCCs ranging from zero to thousands. A previous study of sun exposure and numbers of BCCs found that the BCC site distribution in NBCCS patients differed from that in the general population.[12] While 86–88% of BCCs in the general population occur on sun exposed areas of the body, only 50–65% of BCCs in NBCCS patients occur in such areas, suggesting that frequent sun exposure is not essential for the development of BCCs in NBCCS patients. The study also observed little relation between total numbers of BCCs and reported hours of lifetime sun exposure.[12]

Persons in the general population with a BCC are at increased risk for developing an additional BCC. This risk depends on the number of tumours, the five year risk of developing additional BCCs being 27% in patients with a single tumour versus 90% in patients with 10 or more tumours.[13] Several case–control studies have examined the relation between the presence of multiple BCCs and polymorphisms in some metabolic genes of the glutathione-S-transferase (GST) and cytochrome p450 (CYP) families. The results showed that GSTM1 null, GSTT1 null, GSTP1 Val^{105}/Val^{105}, CYP1A1m1, and CYP2D6 extensive metaboliser (EM, wild type allele combinations of CYP2D6*3, 4, and 5) were associated with increased numbers of BCCs.[14–17] In addition, GSTT1 null and CYP2D6 EM were significant determinants of tumour accrual while CYP1A1 Ile/Ile was associated with slower accrual.[15]

These findings led us to hypothesise that polymorphisms in genes, such as GSTs and CYPs, may contribute to the development of BCCs in NBCCS family members. We therefore examined whether allelic variants of these genes contributed to the variation in numbers of BCCs observed in such families.

Abbreviations: BCC, basal cell carcinoma; BPDE, benzo(a)pyrene diol epoxide; GEE, generalised estimating equations; GST, glutathione-S-transferase; NBCCS, naevoid basal cell carcinoma syndrome; PAH, polycyclic aromatic hydrocarbon

Table 1 Demographic and clinical characteristics in 152 members from 13 NBCCS families*

	Affected with NBCCS (n = 69)				Unaffected (n = 83)			
Mean age at examination (years) (range)	38 (0.4 to 87)				41 (4 to 87)			
Sex								
Male	31 (44.9%)				38 (45.8%)			
Female	38 (55.1%)				45 (54.2%)			
Skin type†	1–2	3–4	6	Unknown	1–2	3–4	6	Unknown
	21	31	11	6	9	21	9	44
Clinical features	Present	Absent		Unknown	Present	Absent		Unknown
BCC	50	18		1	2	81		0
Pits (palm/sole)	57	10		2	3	77		3
Jaw cyst	53	14		2	0	80		3

*Only numbers of subjects, not percentages, are presented in the table for skin type and clinical features.
†Skin type: 1–2: always/often burn; 3–4: rarely burn/mostly tan; 6: never burn/always tan moderately or darkly (all subjects in this category were African American).
NBCCS, naevoid basal cell carcinoma syndrome.

METHODS

Study population

The study involved 152 members (69 affected and 83 unaffected) of 13 families with two or more NBCCS cases who were referred by health care professionals or through self referrals and were previously evaluated at the National Institutes of Health (NIH), beginning in 1985. The family members were clinically evaluated and blood samples were collected under institutional (National Cancer Institute (NCI)) review board approved protocols. A diagnosis of NBCCS was made if a person had any of the following: two major criteria (multiple BCCs or one BCC in a person younger than 20 years of age, jaw cysts, and palmar and/or plantar pits); one major criterion and two minor criteria (lamellar calcification of the falx cerebri, rib anomalies, medulloblastoma, ovarian fibroma, flame shaped lucencies in the phalanges); a family history of NBCCS and one major criterion; or a family history of NBCCS and two minor criteria. Current numbers of BCCs were assessed at the time of the clinical examination. Cumulative numbers of past BCCs (excluding current BCCs) were obtained through self report. Total lifetime BCC numbers were derived by adding the current and past numbers of BCCs. Numbers of pits were determined at examination and numbers of jaw cysts were obtained through self report.

Genotyping

Genotyping of CYP1A1m1 (CYP1A1*2A; rs4646903), CYP1A1m2 (CYP1A1*2C; rs1048943), CYP2D6*4 (G to A transition at intron 3/exon 4), GSTM1 deletion, GSTT1 deletion, GSTP1 Val^{105} (GSTP1*B; rs947894), and GSTP1 Val^{114} (rs1799811) was done (under contract) by Albany Molecular Research (Bothell, Washington, USA) using Taqman genotyping analyses developed on the basis of published sequences.

Statistical analysis

The absence or presence of each of the clinical features of NBCCS such as BCCs, pits (palm or sole), and jaw cysts was analysed separately as binary outcomes. For the quantitative phenotypes such as numbers of BCCs, pits, and jaw cysts, we restricted our analysis to subjects who had the clinical features, and categorised the numbers in two different ways. First, we divided the numbers of BCCs and pits into two groups, above and below the median ($\leqslant 50$ and >50 for BCCs and $\leqslant 10$ and >10 for pits). For jaw cysts, we created two groups based on whether subjects had single or multiple jaw cysts ($=1$, >1). We did not use the median number of jaw cysts (5) as few CYP1A1 variant carriers had more than one cyst. Second, we categorised numbers of BCCs and jaw cysts into multiple groups (six for BCCs and three for jaw cysts) for a better characterisation of the relation between these quantitative phenotypes and the genetic variants.

We assessed the associations between NBCCS characteristics and genotypes using a generalised estimating equations (GEE) approach to account for correlations among members of the same family.[18] Odds ratios (ORs), 95% confidence intervals (CIs), and tests for trend were computed by using unconditional logistic regression models for binary outcomes (PROC LOGISTIC, SAS 8.2) and by cumulative logistic

Table 2 Genotype frequencies and odds ratios for GSTP1 and GSTM1 polymorphisms in NBCCS family members with BCCs

Variants	BCC ($\leqslant 50$)	BCC (>50)	OR* (binary) (95% CI)	OR§ (ordinal) (95% CI)
GSTP1_105				
Ile/Ile	7 (26.9%)	11 (45.8%)	1	1
Ile/Val	15 (57.7%)	13 (54.2%)	0.36† (0.22 to 0.59)	0.58† (0.34 to 0.88)
Val/Val	4 (15.4%)	0	0.13† (0.05 to 0.35)	0.34† (0.14 to 0.78)
Ile/Val + Val/Val	19 (73.1%)	13 (54.2%)	0.44 (0.23 to 0.84)	0.55 (0.35 to 0.87)
GSTP1_114				
Ala/Ala	21 (79.3%)	24 (100%)		
Ala/Val + Val/Val	6 (20.7%)	0 (0.00)	N/D‡	0.20 (0.05 to 0.88)
GSTM1_del				
wt	17 (55.6%)	9 (37.5%)	1	1
mut	12 (44.4%)	15 (62.5%)	2.08 (0.80 to 5.45)	1.00 (0.44 to 2.28)

Values are n (%).
*Odds ratios and 95% confidence intervals estimated by logistic regression using generalised estimating equations to account for familial correlation. The outcome was analysed as a binary variable ($\leqslant 50$, >50).
†Fitted with trend (see Methods for details).
‡Cannot be estimated because no patients with BCC >50 carried the Val^{114} allele.
§The outcome was analysed as an ordinal variable with categories: 1, 2–9, 10–39, 40–99, 100–199, 200+.
BCC, basal cell carcinoma; CI, confidence interval; NBCCS, naevoid basal cell carcinoma syndrome; OR, odds ratio.

regression for ordinal outcomes (PROC GENMOD, SAS 8.2). The working correlation matrix was chosen to be the independent correlation matrix. In confirmatory analysis for binary outcomes we used conditional logistic regression, conditioning on families (PROC PHREG, SAS 8.2). We adjusted the association for age, sex, and skin type by including these variables in the regression models.

RESULTS

We genotyped 152 members of 13 multiplex NBCCS families (69 affected with NBCCS and 83 unaffected) for seven polymorphisms from five metabolic genes. The clinical characteristics of these subjects are summarised in table 1. Ten of the 13 families (77%) had identified PTCH1 mutations. The NBCCS patients and their unaffected family members were similar with regard to age at examination and sex. Fifty of 69 NBCCS patients (72.5%) versus 2 of 83 (2.4%) unaffected family members had BCCs. The median number of BCCs in the family members with BCC was 44, ranging from 1 to 1000. None of the tested polymorphisms was associated with the NBCCS affection status. In addition, no significant associations were observed between any of the polymorphisms studied and the presence or absence of any of the clinical features of NBCCS, such as BCCs, pits (palm or sole), and jaw cysts.

Table 2 shows the frequencies and odds ratios of some polymorphisms in subjects with large and small numbers of BCCs. This analysis was restricted to subjects with BCCs. Only those allele variants that were associated with numbers of BCCs are presented. CYP1A1m1, CYP1A1m2, CYP2D6*4, and GSTT1 null had similar distributions among subjects with high or low numbers of BCCs. GSTM1 null was observed more often among individuals with more than 50 BCCs; however, the association was not statistically significant (table 2). No GSTP1 Val^{114} (combined Ala/Val and Val/Val) alleles or GSTP1 Val^{105}/Val^{105} (homozygous genotypes) were observed among subjects with BCC >50. Results obtained from the logistic regression model showed that the association between the GSTP1 Val^{105} allele (combined Ile/Val and Val/Val) and numbers of BCCs was significant (OR = 0.44 (95% CI, 0.23 to 0.84)). In addition, the variant allele (Val^{105}) showed a dosage dependent effect ($OR_{Ile/Val} = 0.36$ (0.22 to 0.59); $OR_{Val/Val} = 0.13$ (0.05 to 0.35); fitted with trend) (table 2). Odds ratios for GSTP1 Val^{114} using the binary classification could not be estimated as this allele was not observed in the group with BCC >50. However, the ordinal model yielded significant results (OR = 0.20 (0.05 to 0.88)). The results of the ordinal logistic regression models were similar to those obtained from the binary models for GSTP1 Val^{105}. The suggestive association between GSTM1 null and large BCC numbers obtained in the binary model was not observed in the ordinal multinomial model (table 2). Adjustment for age, sex, and skin type did not alter the odds ratios or significance levels (data not shown). Similar results were obtained when GSTP1 Val^{105} and Val^{114} were combined (binary model: $OR_{Val\_105\ or\ Val\_114} = 0.34$ (0.21 to 0.53); $OR_{Val\_105\ and\ Val\_114} = 0.11$ (0.05 to 0.28)).

We also examined the effect of the seven polymorphisms on the numbers of pits (palm or sole) and the numbers of jaw cysts. No significant associations between any of the polymorphisms genotyped in this study and numbers of pits were identified. However, three cytochrome p450 polymorphisms (CYP1A1m1, CYP1A1m2, and CYP2D6*4) all showed protective effects on jaw cyst numbers when jaw cysts were modelled as either a binary or an ordinal variable (table 3). Adjustment of covariates did not change the results significantly, but risks associated with CYP1A1m2 and CYP2D6*4 were stronger after adjustment for skin type ($OR_{CYP1A1m2} = 0.20$ (95% CI, 0.06 to 0.65); $OR_{CYP2D6*4} = 0.27$ (0.16 to 0.45); ordinal model).

Associations between numbers of BCCs or jaw cysts and genetic polymorphisms remained significant after correction for multiple testing based on the false discovery rate (FDR) method.[19] We also modelled numbers of BCCs and jaw cysts using conditional logistic regression, matching on families, as a confirmatory analysis. Odds ratios and 95% confidence intervals obtained from the conditional logistic regression models were similar to those obtained from the unconditional logistic regression analyses using GEE (data not shown).

DISCUSSION

Previous studies have shown that genetic polymorphisms of some enzymes in the cytochrome p450 (CYP1A1, CYP2D6) and GST families (GSTM1, GSTP1, GSTT1) influenced tumour numbers and tumour accrual in patients with BCCs.[15 17 20 21] As BCC is a major clinical feature among NBCCS patients who have varied susceptibility to BCC development, we hypothesised that polymorphisms in these genes might contribute to the variation of BCC numbers in NBCCS patients and their families. The results from our study showed that GSTP1 variants (Val^{105} and Val^{114}) were inversely associated with BCC numbers. In addition, polymorphisms in CYP1A1 and CYP2D6 were associated with fewer jaw cysts. To our knowledge, this study is the first to examine the role of metabolic genes on the numbers of BCCs and jaw cysts in patients from multiplex NBCCS families.

Table 3 Genotype frequencies and odds ratios for CYP1A1 and CYP2D6*4 polymorphisms in NBCCS family members with jaw cysts

Variants	Jaw cyst (=1)	Jaw cyst (>1)	OR* (binary) 95% CI	OR† (ordinal) 95% CI
CYP1A1_m1				
wt	5 (55.6%)	33 (80.5%)	1	1
mut	4 (44.4%)	8 (19.5%)	0.30 (0.11 to 0.85)	0.27 (0.12 to 0.58)
CYP1A1_m2				
wt	8 (88.9%)	38 (92.7%)	1	1
mut	1 (11.1%)	3 (7.3%)	0.63 (0.05 to 8.23)	0.25 (0.08 to 0.78)
CYP2D6*4				
wt	6 (66.7%)	31 (75.6%)	1	1
mut	3 (33.3%)	10 (24.4%)	0.65 (0.22 to 1.89)	0.33 (0.18 to 0.60)

Values are n (%).
*Odds ratios and 95% confidence intervals estimated by logistic regression with familial correlation adjusted by generalised estimating equations. The outcome was analysed as a binary variable (=1, >1).
†The outcome was analysed as an ordinal variable with three categories (1, 2–4, ⩾5).
CI, confidence interval; NBCCS, naevoid basal cell carcinoma syndrome; OR, odds ratio.

The protective mechanism of GSTP1 Val allele variants against multiple BCCs in the NBCCS families is unclear. GSTP1 detoxifies several potential carcinogens, including benzo(a)pyrene diol epoxide (BPDE), and polycyclic aromatic hydrocarbons (PAHs).[22 23] GSTP1 exon 5 (Ile105Val) and exon 6 (Ala114Val) variants have functional effects on the GST gene product, resulting in different catalytic activities for different mutagens and carcinogens that may differentially affect cancer susceptibility.[23–25] The Val^{105} allele was shown to metabolise some PAHs more efficiently than the Ile^{105} allele and to possess up to a fivefold greater enzymatic activity towards the active BPDE than Ile^{105},[23 24] suggesting that the Val allele may be related to a reduced cancer risk with certain carcinogen exposures. In fact, GSTP1 Ile^{105} has been associated with an increased risk of prostate cancer[26] and greater numbers of squamous cell carcinomas compared with Val^{105}.[27] Ramachandran *et al* observed the same protective effect of GSTP1 Val^{105} and Val^{114} on BCC numbers in patients with BCC, but only in the subgroup of patients with a single new lesion at any presentation.[17] In the same study, GSTP1 Val^{105}/Val^{105} was associated with increased BCC numbers in BCC cases with the multiple presentation phenotype, although the number of subjects in that group was quite small. Limited by the sample size, we did not have adequate power to analyse these two phenotypes separately. In contrast to the findings reported by Ramachandran *et al* and Lear *et al*,[15 17 20 21] we did not observe significant associations between polymorphisms of CYP1A1m1, CYP2D6, or GSTT1 and BCC numbers. Future studies with larger sample sizes will be needed to resolve these discrepancies.

Other studies have previously suggested that age, sex, and skin type may influence BCC numbers.[21 28] The role of ultraviolet light exposure, the main causative factor in BCC pathogenesis, in determining numbers of BCCs is unclear. We did not previously observe a strong relation between numbers of BCCs and lifetime sun exposure in 16 NBCCS multiplex families,[12] 11 of which are included in the current study. Thus we did not adjust for sun exposure in our study when modelling the associations between the genetic variants and BCC numbers. We did adjust for age, sex, and skin type as possible confounding variables; however, the results were quite similar to those without adjustments.

The major limitation for this study was the small sample size. This limitation precluded adjustment for PTCH1 mutations as each family had a different mutation. In addition, we did not have sufficient power to assess gene–environment and gene–gene interactions. Another limitation of our study is that the numbers of BCCs and jaw cysts were partly obtained from self report. In addition, given the length of the study (10 years), individuals who were examined early in the course of the study might have developed additional BCCs or jaw cysts after the initial examination. Therefore, absolute counts may not be accurate. To reduce the potential bias, we categorised counts into reasonably large classes rather than analysing actual reported numbers, though the latter approach may be more powerful.

In summary, our results need to be interpreted with caution, and larger studies are needed to confirm the findings. However, this exploratory study is the first to examine the role of genetic variants in the development of BCCs in NBCCS families. Our results are consistent with previous findings in sporadic BCC, that variants in some metabolic genes may be important in determining BCC numbers. In addition, our study identified some genetic variants that may be associated with the numbers of jaw cysts in NBCCS patients. These preliminary findings, if confirmed, will yield important clues to understanding the aetiology of NBCCS.

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